UNIVERSITI PUTRA MALAYSIA

CORRELATION BETWEEN VAGINAL CYTOLOGY, SERUM PROGESTERONE AND OESTRADIOL-17B IN CAPTIVE SUMATRAN RHINOCEROS (*DICERORHINUS SUMATRENSIS)*

CHOONG SIEW SHEAN

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CORRELATION BETWEEN VAGINAL CYTOLOGY, SERUM PROGESTERONE AND OESTRADIOL-17β IN CAPTIVE SUMATRAN RHINOCEROS (*DICERORHINUS SUMATRENSIS*)

By

CHOONG SIEW SHEAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Veterinary Science

March 2002

DEDICATION

To whom work towards the conservation of the Sumatran rhinoceros.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

CORRELATION BETWEEN VAGINAL CYTOLOGY, SERUM PROGESTERONE AND OESTRADIOL-17β IN CAPTIVE SUMATRAN RHINOCEROS (*DICERORHINUS SUMATRENSIS*)

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March 2002

Chairman: **Rosnina Hj Yusoff, D.V.M., M.S., Ph.D.**

Faculty: **Veterinary Medicine**

Vaginal cytology was conducted in 4 females for 121 to 204 days, and 328 vaginal smears were examined. Vaginal flushing and aspiration were done to obtain vaginal cells, and then stained according to Modified Shorr's staining method. Blood samples were also collected for hormonal analysis, so as to be used as a guide in the determination of the different stages of oestrous cycle. Cellular morphology and staining properties were similar to those reported previously in bitches and cows. The percentage of cornified cells was found to be significantly different from other cell types on the day of oestrus. A total of 12 eosinophilic index (EI) peaks were recorded, averaged at 57.42±12.96%; with the highest at 88.17%, and the lowest at 49.10%. Twenty five percent of EI peaks observed were of second peaks, ranged between 49.06% and 65.00 %, and occurred 13.33±2.08 days after the first peak. Length of

oestrous cycle was 22±6.36 days based on vaginal cytology. From the serum progesterone (P_4) profile, the recorded length of oestrous cycle was 28±7.64 days. It was also noted that oestrous cycles were irregular and inconsistent during this study period, lag periods between two oestruses ranged between 21 and >100 days. The range of serum P_4 concentration differed between individuals. The highest concentration recorded was 3.47 ng/mL in one individual; whereas the lowest peak recorded was only 1.34 ng/mL. However, the effect of such differences on the reproductive functions of these animals is yet to be determined. Correlation of EI with serum P_4 profile was weak, with correlation coefficient, r, valued at -0.254. Nevertheless, correlation coefficient in one individual (R_2) was higher, with r = -0.408. This may be due to variations in the level of EI and progesterone concentrations among the animals and between oestrous cycles of an animal. Serum oestradiol-17β (E_2) profile was erratic and did not correlate significantly with either vaginal cytology or serum P_4 profile. Wide range of serum E_2 between individuals was also documented, with the highest and lowest values being 28.00 pg/mL and 6.49 pg/mL respectively. Peaks of E_2 occurred throughout the length of oestrous cycle indicated by serum P_4 profile. This may be due to the low specificity and sensitivity of the commercial kit used towards oestradiol-17β detection in the Sumatran rhinoceros. This study showed that there are reservations in the use of vaginal cytology for detection of oestrus in this species of animal.

Abstrak tesis yang dikemukakan kepada Senat Univerist Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

KORELASI ANTARA SITOLOGI VAGINA, PROGESTERON DAN ESTRADIOL-17β DALAM BADAK SUMBU DALAM KURUNGAN (*DICERORHINUS SUMATRENSIS*)

Oleh

CHOONG SIEW SHEAN

March 2002

Pengerusi: **Rosnina Hj Yusoff, D.V.M., M.S., Ph.D.**

Fakulti: **Perubatan Veterinar**

Sitologi vagina pada empat ekor badak sumbu (*Dicerorhinus sumatrensis*) betina telah dijalankan selama 121 hingga 204 hari, dan pemeriksaan 328 calitan sampel vagina telah dilakukan. Sel vagina dikumpul secara pengumbahan dan pengaspiratan, diikuti dengan pewarnaan secara Modified Shorr. Sampel darah juga diambil untuk analisis hormon supaya peringkat kitaran estrus boleh dikenal pasti. Morfologi sel dan kesan pewarnaan sel vagina badak sumbu didapati menyerupai sel vagina anjing dan lembu. Apabila perbandingan antara peratusan jenis sel lupus semasa estrus dibuat, peratusan sel 'cornified' berbeza secara keertian dengan jenis sel yang lain. Sebanyak 12 tahap puncak indeks eosinofil (EI) telah dicatat, puratanya adalah 57.47±12.96%, dimana nilai tertinggi adalah 88.17% dan nilai terendah adalah 49.10%. Dua puluh lima peratus daripada tahap-tahap puncak EI yang tercatat adalah tahap puncak kedua, dimana ianya berlaku 13.33±2.08 hari selepas tahap puncak pertama,

dan julat nilainya adalah antara 49.06% hingga 65.00%. Sitologi sel vagina mendapati bahawa kitaran estrus adalah selama 22.00±6.36 hari. Walau bagaimanapun, berasaskan pada keputusan analisis hormon progesteron (P_4), kitaran estrus adalah selama 28.00±7.64 hari. Di samping itu, kitaran estrus juga didapati tidak mengggambarkan corak yang sepatutnya semasa kajian ini dijalankan, ekoran terdapatnya peringkat berehat selama 21 hingga lebih daripada 100 hari. Julat kepekatan P_4 serum berbeza antara individu haiwan yang dikaji. Kepekatan tertinggi adalah 3.47 ng/mL, manakala nilai tinggi yang terendah adalah 1.34 ng/mL. Akan tetapi, kesan perbezaan dalam kepekatan hormon ini pada pembiakan haiwan tersebut belum diketahui. Korelasi antara EI dan P_4 serum adalah rendah, dimana koefisien korelasi, r, yang diperolehi bernilai -0.254. Namun, korelasi EI dan P_4 pada salah satu individu (R_4), r yang lebih tinggi, -0.408, diperolehi. Keadaan ini mungkin disebabkan oleh perbezaan dalam nilai EI dan kepekatan P_4 antara individu yang berlainan serta antara kitaran estrus yang berbeza pada individu itu tersendiri. Oestradiol-17β (E_2) serum adalah eratik dan tidak mempuyai korelasi yang signifikan dengan EI mahupun P_4 serum. Nilai kepekatan tertinggi E_2 antara individu juga mempunyai julat yang agak besar, dimana nilai tertiggi dan terendah masing-masing adalah 28.0 pg/mL dan 6.49 pg/mL. Selain daripada itu, catatan mengenai tahap puncak E_2 telah dibuat pada hampir setiap peringkat kitaran estrus. Situasi in besar kemungkinan disebabkan oleh kurangnya spesifikasi dan ketidakpekaan kit komersial yang digunakan untuk mengesan E_2 pada badak sumbu. Pada keseluruhannya, keberkesanan sitologi vagina dalam mengesan estrus haiwan spesis ini masih mampu dipertikaikan.

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I certify that an Examination Committee met on 12th March 2002 to conduct the final examintation of Choong Siew Shean on her Master of Veterinary Science thesis entitled "Correlation between Vaginal Cytology, Serum Progesterone and Oestradiol-17β in Captive Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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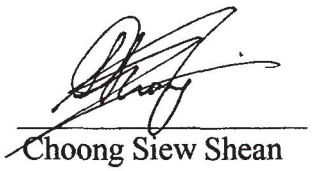
Date: 13 JUN 2007

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



Choong Siew Shean

Date: 20 MAC 2002

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LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS

AI	Artificial insemination
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
E_1	Oestrone
E_2	Oestradiol
EI	Eosinophilic index
EIA	Enzymeimmunoassay
FSH	Follicle stimulating hormone
GCMS	Gas chromatography mass spectrometry
HPLC	High performance liquid chromatography
KPI	Karyopyknotic index
LH	Luteinizing hormone
P_4	Progesterone
RIA	Radioimmunoassay
SCI	Superficial cell index
SG	Specific gravity
WWF	World Wide Fund for Nature

CHAPTER I

INTRODUCTION

Once there were hundred of species of rhinoceros roaming the earth. Their home range covers the swamps in Asia, to the woodlands and savannah of the Sahara, over the forests of Europe and North America, and even in the desert of northwestern Namibia in Africa. They were the most tenacious animals living at that period of time. However, only five species of rhinoceros exists today, and all are threatened with extinction. These five species are Africa's black rhinoceros *(Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*), Asia's Javan rhinoceros (*Rhinoceros sondaicus*), the Sumatran rhinoceros (*Dicerorhinus sumatrensis*), and the Indian rhinoceros (*Rhinoceros unicornis*).

The present predicament status of the rhinoceros is partly due to habitat destruction and land conversion. However, the major cause of death was driven by the demand for rhinos' horns and other parts for use in traditional Chinese medicine, and in North Yemen horns were carved into decorative jambia (traditional dagger) handles. During the 1970s alone, 50% of the world's remaining rhinos disappeared. At the height of the massacre in the 1970s and early 1980s, six Asian countries - China, North Yemen, Taiwan, Hong Kong, Japan and South Korea - were buying up most of the rhino horns. China holds by far the biggest recorded stockpile of rhino horns in the world, weighing over four tonnes (World Wide Fund for Nature, 1996).

The Sumatran rhinoceros, the only species found in Malaysia, is the smallest, hairiest and one of the rarest among the five species that exists today. It was listed on the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1977, and hence international trade on this species is prohibited. These animals weigh around 550 to over 800 kg, and stand about 150 cm tall at the shoulder. Their bodies are covered with thick and rough skin, folded at the shoulder, pelvis and lower abdominal area. Hairs are mostly found tufting the ears, at dorsal side of the body, external genitalia and tip of the tail. Two horns are distinctively located at the dorsum of their nose, with the anterior one being larger and longer.

In the year 2000, it is estimated that there are less than 300 Sumatran rhinoceros in the world, surviving in very small and highly fragmented populations in Southeast Asia (Fig. 1.1). Between 1985 and 1995, the world population of Sumatran rhinoceros (Fig. 1.2) had declined tremendously by 50% and this trend appears to be continuing into the new millenium (International Rhino Foundation, 2000). The current wild population in Peninsular Malaysia stands at 40-48 individuals, almost half of what existed in 1989 (estimated at 67 to 109 animals). The major threats faced by this species of animals are severe habitat destruction and fragmentation and segregation of breeding population due to encroaching of land for development by human beings. Probably because of the low number of surviving Sumatran rhinoceros within the jungle of Peninsular Malaysia, chances of sighting these animals are very slim, let alone poaching. Therefore poaching is less of a threat

to these animals compared to that of environmental disruption mentioned above (Zainal-Zahari, pers. comm.).

Realising the danger of extinction faced by the Sumatran rhinoceros in the wild, the Department of Wildlife and National Parks have embarked on conservation programmes in the 1980's, including captive breeding to prevent such a tragedy from occurring. However, captive breeding is yet to be successful. Active breeding for the past year or so at the Rhinoceros Conservation Centre in Sungai Dusun, Selangor had resulted only in one pregnancy that unfortunately ended with abortion at the first month of pregnancy.

Aggressive courtship behaviour may be the major contributing factor that causes difficulty in breeding of Sumatran rhinoceros. Such occasion usually occurs due to untimely introduction of the male to a non-oestral female. In order to determine oestrus, various methods such as plasma/serum hormonal assays, faecal metabolites assay and ultrasonography had been employed. These methods may prove to be effective, but are usually costly, tedious and require professional handling.

In view of the dilemma confronted earlier, an easy, economical and most importantly, non-invasive way to detect oestrus in the Sumatran rhinoceros is needed. Vaginal cytology was found to fit all three of the criteria mentioned above. Thus the main objective of this study is to determine the significance of vaginal

cytology that is to be used to identify the different stages of oestrous cycle in the Sumatran rhinoceros. Therewith, the null hypothesis, H_O, states that different vaginal cell types do not have a correlation with different stages of oestrous cycle in the Sumatran rhinoceros. While the research hypothesis, H_A, states that different vaginal cell types do have a correlation with the different stages of oestrous cycle in the Sumatran rhinoceros. It is hoped that vaginal cytology can be utilized in predicting the oestrous cycle of Sumatran rhinoceros and thus, increase the possibility of successful captive breeding.

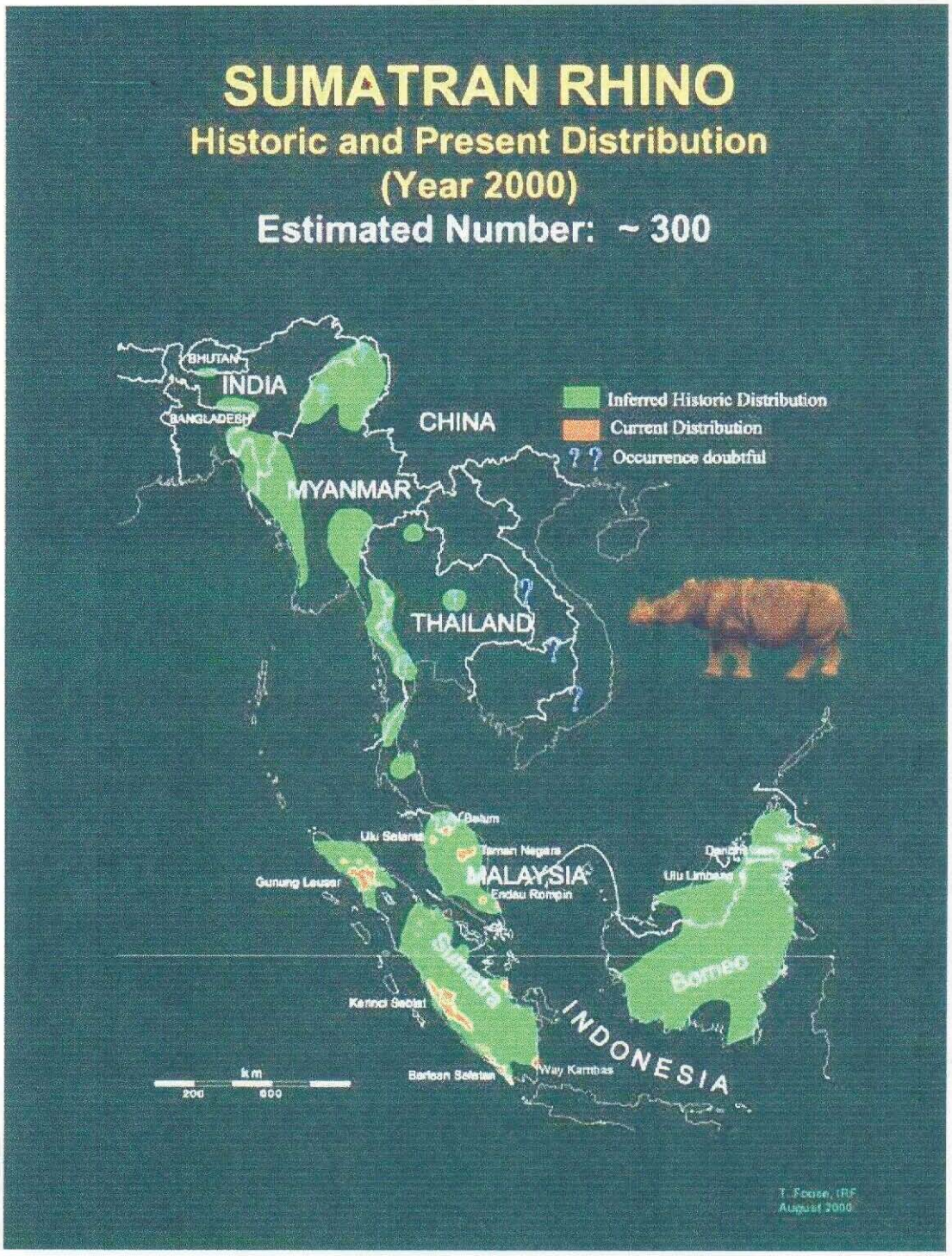

Figure 1.1: World distribution of Sumatran rhinoceros
Source: Foose, T. 2000. http://www.Rhinos-irf.org/rhinos/sumatran.html.

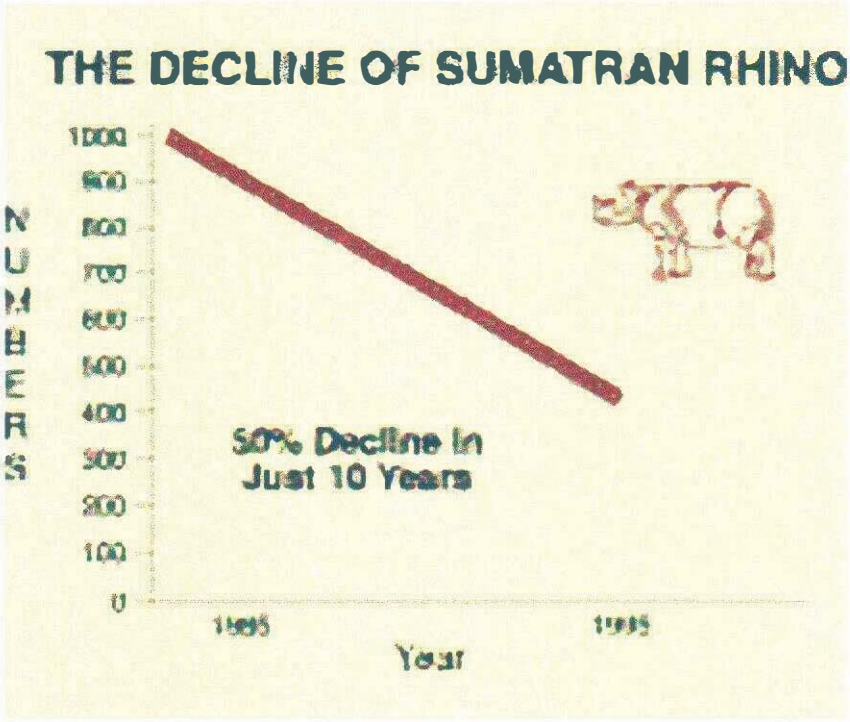

Figure 1.2: Decline in world population of Sumatran rhinoceros (1985-1995)
Source: International Rhino Foundation. 2000.
http://www.Rhinos-irf.org/rhinos/sumatran.html.

CHAPTER II

LITERATURE REVIEW

2.1 Anatomy of the Female Sumatran Rhinoceros Reproductive Tract

In a live animal, the labium is an elongated vertical structure, with a deep convex groove on either side of the vulva opening. On the average, its width and length in an adult rhinoceros are 8.1 cm and 6.9 cm respectively. Vulva lips are thick, wrinkled and appear greyish to grey. The density of coarse hairs covering the vulva lips varies among individuals. The dorsal commissure is rounded while the ventral commissure tapers to form a convex structure with a slight caudal protrusion. The clitoral fossa is situated 2 to 3 cm cranially from the ventral commissure, with a deep central depression coursing cranially from the former. The clitoris is short and broad, measuring 1.0 to 1.5 cm in diameter, and is flattened dorsoventrally. The glans forms a pointed projection over the clitoral fossa (Zainal-Zahari, 1995).

Ultrasonography reveals that the vagina is situated dorsal to the urinary bladder, primarily extending along the neck of the bladder (the inflated bladder is distinguished by its thin wall and hyperechogenic contents). It is viewed as a mass of tissue, 2.5 to 3.0 cm thick, decreases to 1.7 cm immediately beyond the neck of the bladder. In autopsied animals, the vagina is 17.0 to 18.5 cm long and 5.3 to 7.0 cm in diameter, consisting of a thick muscular coat (Zainal-Zahari, 1995).

The cervix is located immediately over the brim of the pelvis usually dorsocranial to the urinary bladder. Its ultrasonographic images are 5.6 to 8.0 cm in length and 4.0 to 5.0 cm in depth and width. (Schaffer *et al.*, 1994). It consisted of a very dense series of alternating annular folds, reflected as hyperechogenic and hypoechogenic contours. On cross section, the cervix tapers from 5.0 cm to 1.5 cm, craniocaudally. The annular folds start caudally as simple elongated interlocking projections that progressively become more inter-twined around the cervical canal. All cervixes examined were tightly closed (Zainal-Zahari, 1995).

2.2 Reproductive Physiology in Sumatran Rhinoceros

Based on plasma progesterone levels, the oestrous cycle of Sumatran rhinoceros averaged 21 days (Zainal-Zahari, 1995). Although oestrus was not determined by sexual receptivity towards a male, the cyclic pattern of plasma progesterone that reached basal levels was highly suggestive of oestrus as in most domestic animals (Christie *et al.*, 1972; Noakes, 1979; Olson *et al.*, 1984a; Concannon, 1986; Miroud & Noakes, 1990), also in other species of rhinoceroses (Hindle *et al.*, 1990; Hindle *et al.*, 1992; Heistermann *et al.*, 1998). On the other hand, reports had indicated that cycle lengths based on changes in female behaviour were longer and showed wide variations: 38 to 58 days in black rhinoceros (Yamamoto, 1967); 17 to 60 days in Indian rhinoceros, (Tong, 1961). Latest work by Heistermann *et al.* (1998) in measuring urinary oestradiol-17β and faecal